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STEREOSCOPIC PHOTOMICROGRAPHY WITH HIGH POWERS

By F. E. IVES

WITH ONE PLATE

The expert microscopist is able more or less perfectly to determine the form of objects with a monocular microscope by focusing successively upon different planes and thus deriving from a series of observations a concrete mental image of the object. The capacity for visualizing a concrete image out of a series of such observations, however, varies greatly with different individuals, depending as it does not only upon the amount of practice with the microscope, but also upon an inherent faculty which some people possess more than others. It may be doubted, however, whether even the most gifted in this respect can generate a concrete mental image of microscopic objects with anything like the certainty and effectiveness with which they are presented by the binocular microscope when using low powers. That binocular microscopes are not more used is no doubt due largely to the fact that only some special forms, difficult of perfect construction and correspondingly costly and troublesome, are adapted for critical work with the higher powers. But even if we admit that the trained microscopist can do very well without binocular vision, we must nevertheless recognize the fact that ordinary photomicrographs with high-power objectives are defective in that they represent as spread out upon a single plane details of structure which the use of the fine adjustment of the microscope readily show to belong to different planes. For instance, in a photograph of *Pleurosigma angulatum* showing white dots and " intercostal markings," the appearance in the photograph would lead one to suppose that it was a representation of a single structure in one plane, whereas in reality the white dots belong to one plane and the " intercostal markings " to another. The use of the fine adjustment shows that this appearance is due to focusing a plane between white dot and black dot, and I have long thought that it should be possible to see this clearly in a binocular microscope adapted to critical work with high powers, and also to show it in a stereoscopic photomicrograph. The latter feat I have recently accomplished, and in the belief that further development and understanding of this method of working may prove of value, I venture to present the results and briefly describe the procedure adopted.

The three examples of stereoscopic photomicrography in high powers which I have to show and the only ones I have so far attempted, were made in a single evening, with the same objective and amplification;—Zeiss 3 mm. apochromatic objective, 18 compensating eye-piece, 13½ in. fixed-focus camera complete in itself, amplification 1,700, Welsbach light, Cramer isochromatic plates without color screen.

The objects are *Pleurosigma angulatum, Coscinodiscus asteromphalus,* and a *Triceratium.* Only a small portion of each frustule is shown. *Pleurosigma angulatum* was dry mounted, in cover-glass contact, and different parts appear in different focal planes owing to roundness of field of the objective; thus we have white hexagons on one part and white dots on another, with various effects between, where the stereoscope shows structure on two distinctly separate planes. *Coscinodiscus* shows a membrane with lace-like areolations, supported by a thick grid which is in most parts hexagonal. *Triceratium* shows a bossed membrane which appears to be punctured by groups of round holes in parallel rows, and supported by a hexagonal grid.

The first essential to the production of these results is that none of the diffraction pencils which define such minute detail shall be cut off at the back of the objective. The difference between the two elements of the stereogram must be due entirely to differences in the centering of the illuminating cone, and in order to avoid an exaggerated appearance of relief, the separation of the angles of illumination must not be greater than it would be in low power work with an objective of say .30 or .35 n. a., and the central rays should act in both photographs. The angular aperture of the illuminating cone for *Pleurosigma* and *Coscinodiscus* was only about .70 n. a., and was decentered, first to the right, for one photograph, and then to the left, for the other photograph, but altogether so little that half of the illumination was alike for both photographs. For the *Triceratium* I opened the condenser diaphragm until it only just touched the edges of the full dry cone, and then decentered to cover about one quarter of the aperture, first on one side, and then on the other.

The fact that the diffraction image and the dioptric image occupy different planes when the microscope tube length is not correctly adjusted, makes it of vital importance for truthful representation to make such adjustments correctly, and no doubt considerable caution should be used in interpreting the results until further experiment has more positively determined the value and limitations of the method.

EXPLANATION OF PLATE

Plate I

APPARATUS FOR STEREOSCOPIC PHOTOMICROGRAPHY

The camera with which the photographs referred to in the text were made. It is a simple box camera having a lens the focus of which corresponds exactly to the length of the box, and is adjustably mounted on a base fitting against the base of the microscope, in such manner that it may be brought into use in a few seconds, without disturbing the microscope, and removed as a rigid whole by a single rectilinear movement of one hand.

The camera swings from centres concentric with the pivot of the microscope, making it quickly adjustable for inclination, and may be used without even refocusing provided that the microscopist's vision is emmetropic.

For stereoscopic work, two plates are exposed in quick succession, condenser diaphragm decentered to the right for one and to the left for the other, and are then developed together.

PLATE I